



# IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203



Human Journals

**Research Article**

July 2023 Vol.:27, Issue:4

© All rights are reserved by Joju Joseph Kattakayam et al.

## Analytical Methods and Stability Studies for Gastro Retentive Superporous Hydrogels of Glipizide



**IJPPR**  
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203



**Joju Joseph Kattakayam\*<sup>1</sup>, Adkar Prafulla Prakash<sup>2</sup>**

<sup>1</sup>*Research Scholar Sunrise University, Alwar, Rajasthan, India*

<sup>2</sup>*Research Guide Sunrise University, Alwar, Rajasthan, India*

**Submitted:** 30 June 2023  
**Accepted:** 20 July 2023  
**Published:** 30 July 2023

**Keywords:** *UV, RP-HPLC and Bioanalytical method*

### ABSTRACT

The UV, RP-HPLC and Bioanalytical methods were used to estimate glipizide. The mobile phase consisted mixture of 10mM concentration of phosphate buffer (pH adjusted to 2.1) and acetonitrile (34:66) with C18 column (250mm×4.6mm×5μ). The drug retention time was found to be 3.41 min. Representative chromatogram of glipizide monitored at 276 nm. A linear relationship was observed in the concentration range of 1-8 mcg/ml ( $r^2=0.999$ ;  $n= 6$ ). The method was specific as there was no interference at the retention time of the analyte by the blank solution and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4 mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.24-101.12% and 98.88-101.51% for intra and inter-day respectively.



HUMAN JOURNALS

[ijppr.humanjournals.com](http://ijppr.humanjournals.com)

## **INTRODUCTION:**

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patient's need by combining the therapeutic effects of two or more drugs in one product. This presentation will discuss the development and validation of analytical methods (Spectrophotometric & High-performance liquid chromatography (HPLC)) for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

### **Basic criteria for new method development of drug analysis:**

- The drug or drug combination may not be official in any pharmacopeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

### **SYSTEM SUITABILITY <sup>1</sup>:**

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and procedure are capable of providing data of acceptable quality. These tests are used to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

The purpose of the system suitability test is to ensure that the complete testing system (including instruments, reagents, columns, and analysts) is suitable for the intended application.

The parameters that are affected by the changes in chromatographic conditions are,

- Resolution ( $R_s$ ),
- Capacity factor ( $k'$ ),
- Selectivity ( $\alpha$ ),
- Column efficiency ( $N$ ) and
- Peak asymmetry factor ( $A_s$ ).

#### **i) Resolution ( $R_s$ )**

The resolution,  $R_s$ , of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of  $R_s$  is 1.5. It is calculated by using the formula,

$$R_s = \frac{Rt_2 - Rt_1}{0.5(W_1 + W_2)}$$

Where,  $Rt_1$  and  $Rt_2$  are the retention times of components 1 and 2 and

$W_1$  and  $W_2$  are the peak widths of components 1 and 2.

#### **ii) Capacity factor ( $k'$ )**

Capacity factor,  $k'$ , is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column or TLC plate during an isocratic separation. The ideal value of  $k'$  ranges from 2-10. Capacity factor can be determined by using the formula,

$$k' = \frac{V_1 - V_0}{V_0} \times S$$

Where,  $V_1$  = retention volume at the apex of the peak (solute) and

$V_0$  = void volume of the system.

The values of  $k'$  of individual band increase or decrease with changes in solvent strength. In reverse phase HPLC, solvent strength increases with the increase in the volume of the organic phase in the water / organic mobile phase. Typically an increase in the percentage of the organic phase by 10 % by volume will decrease  $k'$  of the bands by a factor of 2-3.

### iii) Selectivity ( $\alpha$ )

The selectivity (or separation factor),  $\alpha$ , is a measure of the relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where,  $V_0$  is the void volume of the column and  $V_2$  and  $V_1$  are the retention volumes of the second and the first peak respectively.

### iv) Column efficiency (N)

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. The smaller the band spread, higher the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 2000 - 100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16 \frac{Rt^2}{W^2},$$

Where  $Rt$  is the retention time and  $W$  is the peak width.

**v) Peak asymmetry factor (As)**

Peak asymmetry factor, can be used as a criterion of column performance. The peak half width,  $b$ , of a peak at 10 % of the peak height, divided by the corresponding front half-width,  $a$ , gives the asymmetry factor.

$$As = \frac{b}{a}$$

For a well-packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

**VALIDATION <sup>2</sup>:**

Validation is derived from Latin which means “strongness”.

**Validation** is documented evidence that provides a high degree of assurance that a specific process will consistently produce a product that meets its predetermined specifications and quality attributes.

**VALIDATION AS DEFINED BY DIFFERENT AGENCIES**

**1. USFDA:** According to this “Validation” is the process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

**2. WHO:** Defines Validation as an action of providing any procedure, process, equipment, material, activity or system actually leads to the expected results.

**3. EUROPEAN COMMITTEE:** Defines Validation as an action of providing in accordance with the principles of GMP that any procedure, process, material, activity or system actually lead to expected results.

**Benefits of Validation:**

- a. Produces quality products
- b. Helps in process improvement technology transfer, related product validation, failure investigation, and increased employee awareness.

- c. Cost reduction by increasing efficacy, few reject, longer equipment life, production of cost-effective products
- d. Helps in the optimization of process or method.
- e. Regulatory affairs- produces approved products and increased ability to export.

#### **ANALYTICAL METHOD VALIDATION <sup>1</sup>:**

"Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application.

#### **Types of Analytical Procedures to be Validated <sup>3</sup>:**

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Typical analytical parameters used in assay validation according to ICH guidelines:

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Limit of quantitation
6. Linearity
7. Range

8. Robustness

**Category-I:** Analytical methods for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

**Category-II:** Analytical methods for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These methods include quantitative assays and limit tests.

**Category-III:** Analytical methods for determination of performance characteristics (e.g. dissolution, drug release).

The type of method and its intended use dictates which parameters are required to be investigated. They are illustrated in the following Table

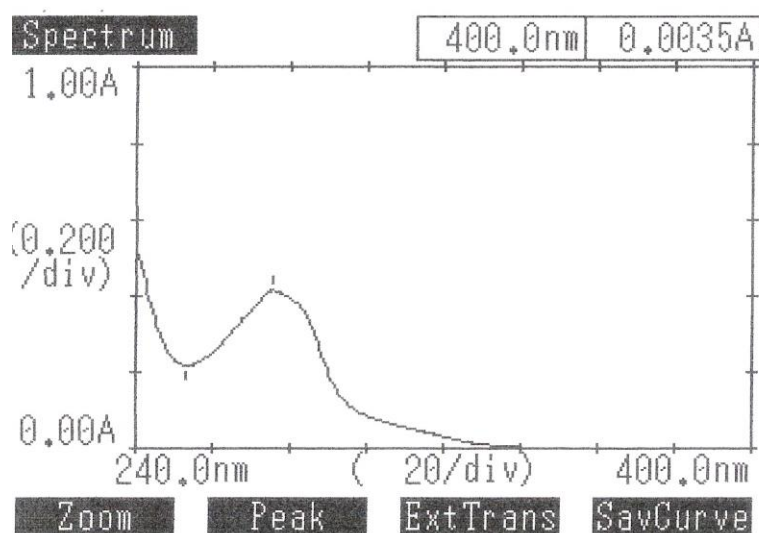
**Data elements required for assay validation**

Analytical Performance Parameters	Assay category-I	Assay Category –II		Assay category –III
		Quantitative	Limit Test	
Accuracy	Yes	Yes	*	*
Precision	Yes	Yes	No	Yes
Specificity	Yes	Yes	Yes	*
LOD	No	No	Yes	*
LOQ	No	Yes	No	*
Linearity and range	Yes	Yes	No	*
Ruggedness	Yes	Yes	Yes	*

**UV Method:** (Method described in USP was followed)

**Stock solution:** 100mg of Glipizide was dissolved in methanol and the volume was made up to 100ml with pH 1.2 to obtain 1mg/ml solution.

**Scanning:** From the stock solution, a 15 µg/ml solution of glipizide was prepared in SGF and scanned between 200-400nm. The spectrum is reproduced in Figure 1. The absorption maxima of 276nm was selected and used for further studies.



**Figure 1: UV spectra of glipizide in pH 1.2**

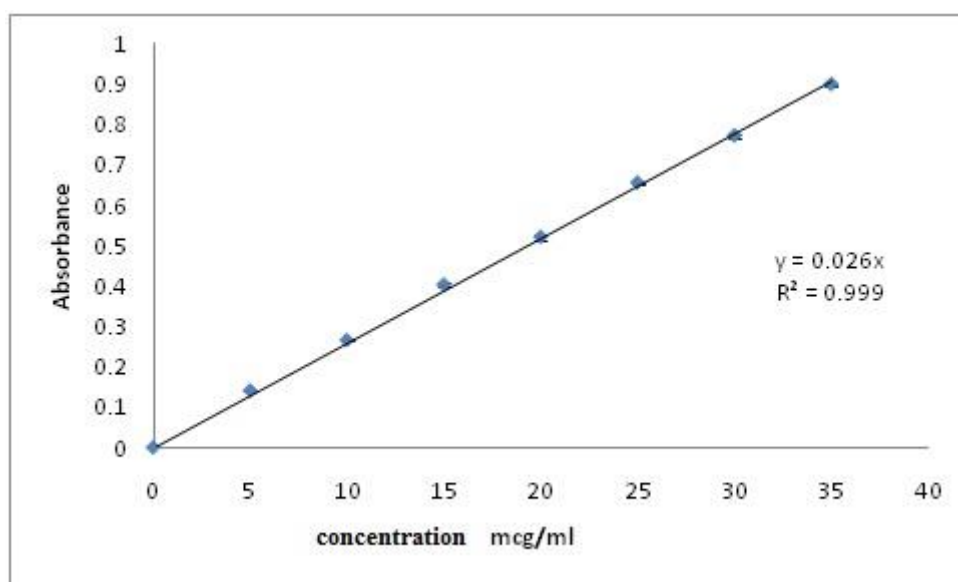
**Standard plot:** From the stock solution (1mg/ml), 10 ml was pipetted and transferred into a 100ml volumetric flask and diluted up to the mark with pH 1.2 buffer solution. From this, aliquots of 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml and 3.5ml were transferred to a series of 10ml volumetric flasks and the volumes were made upto 10ml with pH 1.2 buffer to obtain 5, 10, 15, 20, 25, 30, 35mcg/ml solutions of glipizide respectively. The absorbance of these solutions was measured at 276nm. The data for the standard curve is reported in Table 1. A calibration graph was plotted (Figure 2).



**Table 1: Standard plot data of glipizide in pH 1.2.**

Concentration (mcg/ml)	Absorbance Mean± S.D.*
5	0.140±0.001
10	0.265±0.003
15	0.403±0.003
20	0.521±0.006
25	0.655±0.002
30	0.771±0.003
35	0.899±0.002

\*Standard deviation n=6

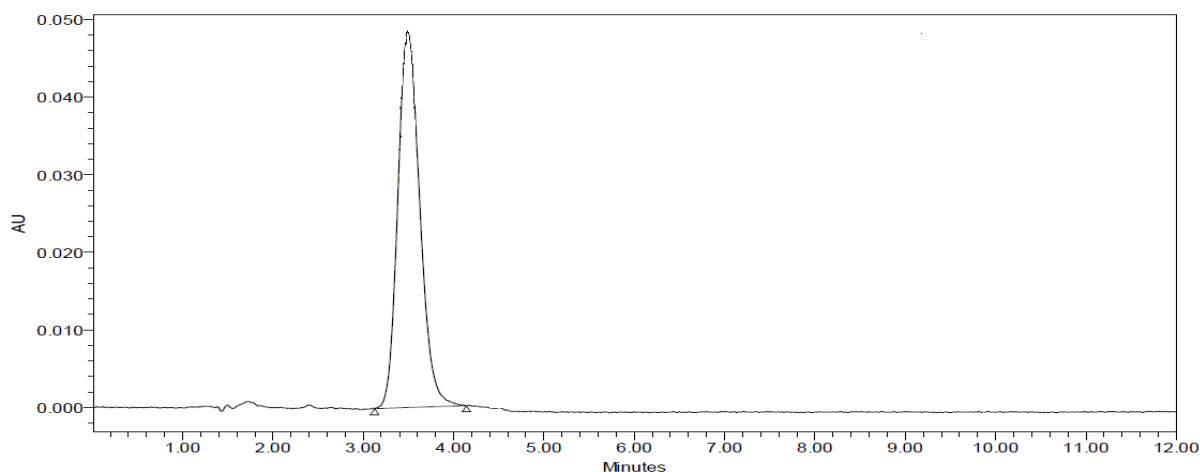


**Figure 2: Calibration curve of Glipizide in pH 1.2**

**HPLC Method: Analytical method**

The RP-HPLC method was used to estimate glipizide. The mobile phase consisted mixture of 10mM concentration of phosphate buffer (pH adjusted to 2.1) and acetonitrile (34:66) with C18 column (250mm×4.6mm×5μ). The drug retention time was found to be 3.41 min. Representative chromatogram of glipizide monitored at 276 nm. A linear relationship was observed in the concentration range of 1-8 mcg/ml ( $r^2=0.999$ ; n= 6). The method was specific as there was no interference at the retention time of the analyte by the blank solution and the

LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4 mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.24-101.12% and 98.88-101.51% for intra and inter-day respectively. The data for the standard curve is reported in Table 2. A calibration graph was plotted (Figure 4).

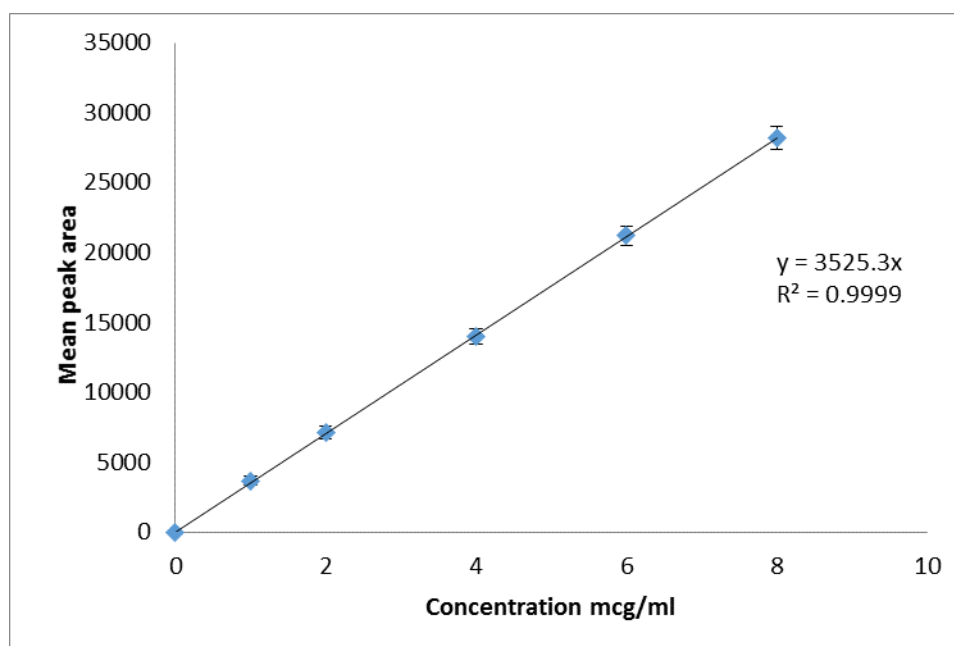


**Figure 3: Typical chromatogram of glipizide by RP-HPLC**

**Table 2: Standard calibration data of glipizide by RP-HPLC**

Concentration(mcg/ml)	Area Mean $\pm$ SD*
1	3662.54 $\pm$ 321.20
2	7112.72 $\pm$ 475.28
4	13997.46 $\pm$ 523.10
6	21205.34 $\pm$ 702.46
8	28182.49 $\pm$ 789.22

\*Standard deviation n=6



**Figure 4: Standard calibration curve of glipizide by RP-HPLC**

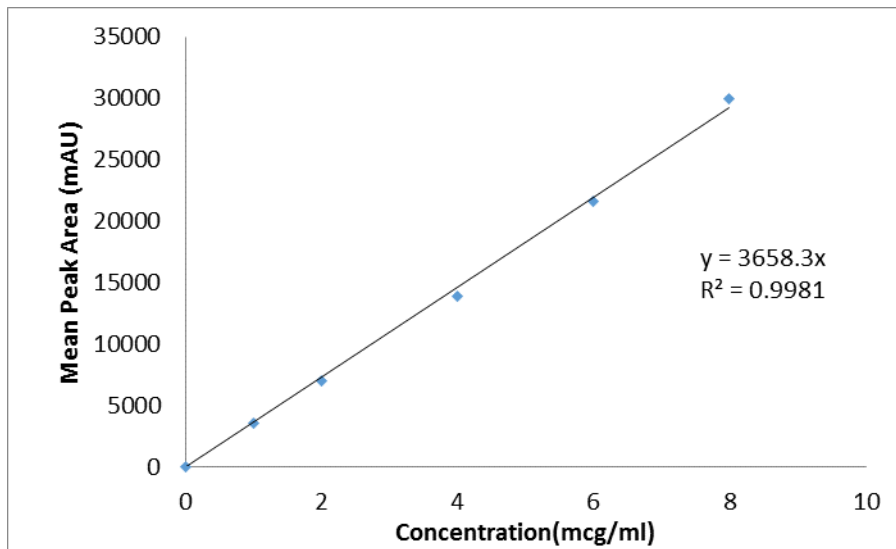
### **Bio analytical method**

The HPLC bio analytical method used for glipizide analysis was rapid and simple. The protein precipitation method was used (Acetonitrile) for extraction, the clear supernatant liquid was separated, filtered ( $0.45\mu$  filter) and injected into the HPLC system. The drug retention time was 3.45 min. A linear relationship was observed in the concentration range of 1-8 mcg/ml ( $r^2 = 0.998$ ;  $n = 6$ ). The results show the linearity between the peak area and the concentration of the analyte. The developed method was specific as there was no interference by the matrix components at the retention time of the analyte and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.04-101.31% and 95.11-103.42% for intra and inter-day respectively. The data for the standard curve is reported in table 3. A calibration graph was plotted (Figure 5).

**Table 3: Standard calibration data of glipizide by RP-HPLC**

Concentration (mcg/ml)	Mean Peak Area Mean $\pm$ SD*
1	3562.23 $\pm$ 200.12
2	7012.42 $\pm$ 323.41
4	13897.88 $\pm$ 868.10
6	21605.92 $\pm$ 901.21
8	29982.65 $\pm$ 769.45

\* Standard deviation: n=6



**Fig 5: Calibration curve for the estimation of glipizide in plasma samples by HPLC method**

**STABILITY STUDIES:**

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized nanoparticle formulation was subjected to stability studies according to ICH guidelines by storing at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH for 6 months. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The observations of accelerated conditions are shown in table 4. Results indicate no significant changes in the parameter even when it was subjected to stress testing for a period of six months.

**Table 4: Stability study data of optimized formulation**

Stability condition	Sampling interval (months)	Physical appearance	% Drug content (mean ± S.D*)
25 $\pm$ 2 $\circ$ C/60 $\pm$ 5% RH	0	No change	98.22 $\pm$ 0.13
	3	No change	$\pm$
	6	No change	$\pm$
30 $\pm$ 2 $\circ$ C/65 $\pm$ 5% RH	0	No change	$\pm$
	3	No change	$\pm$
	6	No change	$\pm$
40 $\pm$ 2 $\circ$ C/75 $\pm$ 5% RH	0	No change	$\pm$
	3	No change	$\pm$
	6	No change	$\pm$

**CONCLUSION:**

All the methods UV, RP HPLC and The HPLC bioanalytical method used for glipizide analysis were rapid and simple and validated as per ICH guidelines. Stability

The protein precipitation method was used (Acetonitrile) for extraction, the clear supernatant liquid was separated, filtered (0.45 $\mu$  filter) and injected into the HPLC system. Drug retention time was 3.45 min. A linear relationship was observed in the concentration range of 1-8 mcg/ml ( $r^2 = 0.998$ ;  $n = 6$ ). The results show the linearity between the peak area and the concentration of the analyte. The developed method was specific as there was no interference

by the matrix components at the retention time of the analyte and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.04-101.31% and 95.11-103.42% for intra and inter-day respectively.

Stability studies are to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH, for the optimized nanoparticle formulation was subjected to stability studies according to ICH guidelines by storing at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH for 6 months. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The observations of accelerated conditions are shown in table 4. Results indicate no significant changes in the parameter even when it was subjected to stress testing for a period of six months.

#### REFERENCES:

1. Tammisetty MO, Challa BR, Puttagunta SB. A novel analytical method for the Simultaneous estimation of remogliflozin and metformin hydrochloride by UPLC/PDA in bulk and formulation. Application to the estimation of product traces. *Turk. J. Pharm. Sci.* 2020 Jun 8;(3)96 -99.
2. Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya UK. Stability indicating liquid chromatographic method for the estimation of remogliflozin etabonate. *Journal of Chemical Metrology.* 2020 Jul 1;14(2):132.
3. Ahmed Gedawy, Hani Al-Salami, and Crispin R Dass. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. *Journal of Food and Drug Analysis.* 2019; 27(1), 315-322.
4. Tripathi KD. 'Essentials of Medical Pharmacology', 5th Edition, Jaypee Brothers Medical Publications (P) Ltd., New Delhi, 2003.p. 167-84.
5. Clarke C. Oral Therapy in Type 2 Diabetes: Pharmacological Properties and Clinical Use of Currently Available Agents. *Diabetes Spectrum.* 1998; 11: 211-21.
6. Ezine Articles [Online]. [Cited 2012 March 05] Available from: URL:[http://EzineArticles.com/?expert=Charlene Nuble](http://EzineArticles.com/?expert=Charlene+Nuble)
7. Arora MM, Chander Y, Rai R. Diabetes mellitus in India- Y2K Not Ok, *Armed Forces Med J India* 2008; 56(1): 1-2.
8. Mohan H. Textbook of Pathology, 4<sup>th</sup> edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi; 2004. p. 805-11.
9. Yoshida R, Sakai K, Okano T, Sakurai Y. Pulsatile drug delivery systems using hydrogels. *Adv Drug Deliv Rev*1993; 11:85-108.
10. Peppas BL, Peppas NA. Dynamic and equilibrium swelling behavior of pH-sensitive hydrogels containing 2-transhydroxyethyl methacrylate. *Biomaterials* 1990; 11:635- 44.
11. Annaka M, Tanaka T. Multiple phases of polymer gels. *Nature*1992; 355:430-2.
12. Firestone BA, Siegel RA. Kinetics and mechanisms of water sorption in hydrophobic, ionizable copolymer gels. *J Applied Polym Sci* 1991; 43:901-14.
13. Dong LC, Hoffman AS. A novel approach for preparation of pH-sensitive hydrogels for enteric drug delivery. *J Control Release* 1991; 15:141-52.
14. Kou JH, Fleisher D, Amidon G. Modeling drug release from dynamically swelling poly (hydroxymethyl methacrylate co-methacrylate acid) hydrogels. *J Control Release* 1990; 12:241-50.

15. Pradny M, Kopecek J. Hydrogels for site-specific oral delivery. Poly[(acrylic acid)-co-(butyl acrylate)] crosslinked with 4,4-di(methacryl amino) azobenzene. Makromol Chem 1990; 191:1887-97.
16. Siegel RA, Firestone BA. pH-dependent equilibrium swelling properties of hydrophobic poly electrolyte co-polymer gels. Macromolecules 1988; 21:3254-61.
17. Kono K, Tabata F, Takagishi T. pH responsive permeability of poly (acrylic acid)-poly (ethylenimine) complex capsule membranes. J Membr Sci 1993; 76: 233-43.
18. Balamuralidhara V, Pramodkumar TM, Srujana N, Venkatesh MP, Vishal G, Krishna KL, Gangadharappa HV. pH sensitive drug delivery systems: A review. Am J Drug Dis Dev 2011 ; (1): 24-48.
19. Ichigawa M, Watanabe S, Miyake Y. A new multiple-unit oral floating dosage system. I: Preparation and in vitro evaluation of floating and sustained-release characteristics. J Pharm Sci 1991; 80:1062-6.
20. Jain SK, Awasthi AM, Jain NK, Agrawal GP. Calcium silicate based microspheres of repaglinide for gastro retentive floating drug delivery: preparation and in vitro characterization. J Control Release 2005; 107:300-9.
21. Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resins containing tranilast and their drug release characteristics (*in vivo*). J Control Release 1991; 16:279-290.
22. Streubel A, Siepmann J, Bodmeier R. Multiple unit gastroretentive drug delivery systems: a new preparation method for low-density microparticles. J. Microencapsul 2003; 20: 329-347.